Table V: Relative rates of incorporation of complementary and non-complementary rNTPs on homopolymeric templates by w.t. and mutant polymerases

I. Template: poly(dC)					
	W.T.	**Y639F**	**G640A**	**Y639A**	**Y639S**
GTP/UTP	>2000 (Mg^{++}) 53 (Mn^{++})*	>1760 55	>1320 n.d.	>184 n.d.	>50 n.d.
GTP/CTP	400 32	550 40	508 n.d.	>184 n.d.	>50 n.d.
GTP/ATP	233 9.3	338 8	388 n.d.	>184 n.d.	>50 n.d.
II. Template: poly(dT)					
	W.T.	**Y639F**	**G640A**	**Y639A**	**Y639S**
ATP/GTP	170 50	94 27	n.d. n.d.	n.d. n.d.	n.d. n.d.
ATP/UTP	121 21	94 20	n.d. n.d.	n.d. n.d.	n.d. n.d.
ATP/CTP	>340 77	>94 60	n.d. n.d.	n.d. n.d.	n.d. n.d.

Numbers are averages from 2 experiments and reflect the ratio of the percentages of labeled rNTPs incorporated into RNA in reactions in which unlabeled complementary rNTPs were in great excess (.5 mM) to labeled complementary or non-complementary rNTPs. Templates were at .1 mg/ml. Polymerases were at 10^{-8} M in Mg^{++} buffer and 10^{-7} M in Mn^{++} buffer.

*The upper number refers to results obtained in Mg^{++} buffer, the lower number to results in Mn^{++} buffer. n.d.: G640A, Y639A, Y639S were poorly active in Mn^{++} buffer, or on poly(dT) under all conditions.

Table VI: Kinetic Constants for Y639F and the W.T. polymerase with rNTPs or dNTPs

		rATP	rITP	dTTP	dUTP	dCTP	dGTP	dATP
Y639F:	K_m	.063-.125	.034-.094	.038-.059	.052-.092	.92-1.6	.185-.264	.20-.35
	k_{cat}	150-210 s^{-1}	180-200 s^{-1}	70-110 s^{-1}	70-130 s^{-1}	50-90 s^{-1}	30-60 s^{-1}	50-70 s^{-1}
W.T.:	K_m	034-.068	029- 059	.209-.262	4 4-9.0	4.3-13.5	.602-.701	2.0-5.0
	k_{cat}	190-220 s^{-1}	170-230 s^{-1}	26-29 s^{-1}	25-39 s^{-1}	9-14 s^{-1}	5-9 s^{-1}	6-9 s^{-1}

Numbers give ranges from 3 experiments The template was supercoiled pT75 at 10^{-7} M, and polymerases were at 10^{-8} M.

Table VII: 2',3'-dideoxy NTP preferences of the Y639F mutant

ATP/ddATP	TTP/ddUTP	CTP/ddCTP	GTP/ddGTP
9.0	7.0	10.0	5.0

Numbers reflect the relative specificity (k_{cat}/K_m) for an NTP vs. the corresponding ddNTP. Relative specificities could not be evaluated for the wild-type T7 RNAP because the ddNTPs are such poor substrates, but these relative specificities appear to be at least 150-fold.

Table VIII: Activity of W.T. and Y639 mutants with NTPs containing different 2'-substituents

NTP	W.T.	Y639F	Y639M
UTP	100	95±6.7	50±1.2
2'-NH2-UTP	5.9±.27	12±.41	3.6±.19
2'-F-UTP	3.1±.14	73±2.6	23±.72
2'-dUTP	2.4±.11	46±2.4	11±.46
CTP	100	103±2.3	54±3.9
2'-NH2-CTP	34±.86	60±2.5	21±.38
2'-F-CTP	3.4±.22	63±3.1	47±.70
2'-dCTP	1.6±.16	57±1.7	32±1.1
ATP	100	96±3.0	51±1.3
2'-NH2-ATP	18±.39	21±.75	.92±.035
2'-F-ATP	6.6±.12	50±1.4	9.7±.20
2'-dATP	2.7±.28	40±1.3	3.2±.11

Activity was determined with pT75 as template but with one of the rNTPs replaced with a dNTP or a 2'-modified NTP. The labeling NTP was UTP (in reactions with 2'-modified CTPs or ATPs) or CTP (in reactions with 2'-modified UTPs). Y639F and Y639M represent enzymes with substitutions of the wild-type (W.T.) tyrosine at position 639 by phenyalanine (F) or methionine (M), respectively.